Phoenix Field Station
Technology Branch
Communicable Disease Center
Public Health Service
U. S. Department of Health, Education, and Welfare

Quarterly Report - April, May, and June

Services Provided in Support of the Planetary Quarantine Requirements of the National Aeronautics and Space Administration under Contract R-137

Report No. 14

June 24, 1966

Laboratory for monitoring bacterial contamination of space components (NASA)

1. Efforts were continued to perfect two model systems for evaluating mechanical methods of recovering viable spores from the interior of plastics. Spores of Bacillus subtilis var. niger were incorporated into polymerized methyl methacrylate (lucite) and recovered by dissolving the plastic in acetone. Solvent-resistant membrane filters (Gelman, alpha 6 and 8) were used to recover the spores for enumeration. The conventional technique of placing the filter on an agar surface could not be employed because water distorted the filter. Maximal recovery of viable spores was obtained when the filter was placed directly on the surface of the agar medium while it was still molten (Table 1).

   Spores were incorporated successfully into pieces of plastic formed either by laminating thin sheets of polystyrene with carbon tetrachloride, or by compressing powdered polystyrene into pellets using carbon tetrachloride as an adhesive. Spores were recovered by dissolving the plastic in carbon tetrachloride and passing the solution through a conventional membrane filter (Millipore, HA) for subsequent enumeration. The rate of dissolution of both plastics was enhanced significantly by ultrasonication. Either system provides a valid control for the evaluation of mechanical means of recovering viable spores from the interior of plastics since acetone and carbon tetrachloride are not toxic to test spores.

2. Studies were advanced on the use of ultrasonic energy for removing microbial contamination from surfaces for subsequent enumeration. Metal surfaces (11 x 16 inches) were contaminated artificially with airborne spores of B. subtilis var. niger in an aerosol chamber. The standard cotton swab (swab-rinse) method was employed for enumeration. Tests were done to determine if ultrasonication could be used to replace mechanical agitation for eluting microorganisms from cotton swabs. The results showed that ultrasonication not only consistently recovered more spores than mechanical agitation, but also effected lower coefficients of variation. Similar results were found when swabs were used to remove microorganisms from naturally contaminated floors at randomly selected sites (Tables 2 and 3).

   Most of the preliminary studies on ultrasonication were conducted using a borrowed ultrasonic bath (JPL). This bath was operated at a fixed power setting rated at 150 watts. A new but similar unit (CDC) was obtained for permanent use. The new bath has a power control unit by which the rated power can be adjusted from 0 to 300 watts. Because the tanks are of comparable size, the CDC unit set at 150 watts should, theoretically, be equivalent

to the JPL unit at 150 watts with respect to removing microorganisms from surfaces. In practice, however, it was found that this was not the case. Comparable removal of heat-fixed spores from strips of stainless steel and glass could be achieved in the CDC unit only at a power setting of 300 watts (Table 4). Evaluation of the CDC unit will continue in an effort to delineate factors, other than power, that may affect the removal of microorganisms from surfaces by ultrasonic energy.

A comparison of mechanical agitation and three ultrasonic systems showed that significantly more spores were recovered from glass surfaces by the CDC and JPL ultrasonic baths than by mechanical agitation or an ultrasonic probe (Table 5). During this particular test, no significant differences among the four methods were noted with the stainless steel surfaces. As reported earlier ultrasonication was significantly more efficient than mechanical agitation in recovering spores from frosted glass (Table 6).

Other tests showed that more organisms were recovered from test surfaces when the sample bottles were placed on the bottom of the tank rather than suspended 1 inch from the bottom (Table 7).

3. Earlier tests showed a coating of sodium silicate on stainless steel strips markedly reduced the adhesion of microorganisms (Tables 8, 9, 10). Similar results were obtained when glass was used as the test surface. Slight but significantly higher percentages of recovery were obtained from coated glass slides which had been exposed to airborne spores of B. subtilis var. niger and also natural contamination as a result of handling (Table 11).

4. Further observations were made on the survival of microorganisms on surfaces. Spores of B. subtilis var. niger in an ethanol suspension inoculated on polystyrene granules (7×10^5/gram) showed no significant die-away when stored at 46 percent relative humidity (R.H.) and temperatures of 20 C (4.7×10^5/gram) and 37 C (4.8×10^5/gram) for 143 days. At 46 percent R.H. and 50 C, the population decreased 1.5 logs (1.8×10^4/gram) during the test period (Figure 1). A parallel series of tests, parts of which were reported earlier, showed that when the seeded plastic was stored at zero percent R.H., rapid and significant die-away was noted. At 50 and 37 C no viable spores were detected after only several days of storage (Figures 2 and 3). In subsequent experiments the death rates of spores suspended in water or ethanol, dried on stainless steel, and stored at 50 C for 105 days at either zero or 46 percent R.H. were determined. No significant differences in death rates were noted for the water suspension of spores at both relative humidities. A slight but faster death rate did occur at zero percent R.H. when an ethanol suspension

of spores was used (Figures 4 and 5). However, this die-away was not as fast as when polystyrene was used as the test surface. It was concluded, tentatively, that either toxic materials emanate from the plastic or certain compounds in plastic become toxic at a relative humidity of zero percent. Experiments are in progress to confirm these results and to determine if the same phenomenon occurs with polymerized methyl methacrylate.

5. Studies were continued to compare the levels and types of microbial contamination in industrial clean rooms and hospital operating rooms. The results definitely showed that the level of aerobic mesophilic microorganisms accumulating on stainless steel surfaces in 4 operating rooms was at least 1 log higher (10^5) than in industrial clean rooms and essentially the same as in factory areas (Tables 12 and 13 and Figures 6 and 7). The types of microorganisms which accumulated on surfaces in the operating rooms resembled those found in factory areas in that relatively high numbers of Bacillus spp. molds, and actinomycetes were found and few staphylococci and micrococci. In clean rooms the opposite pattern usually occurs (Table 14).

   Air sampling patterns also showed that the levels of airborne viable particles per cubic foot of air in operating rooms were significantly higher than in clean rooms even with poor environmental control (Clean room A) and were essentially the same as in factory areas (Figures 8 and 9).

6. Further studies were made on the formulation of culture media for optimum recovery of aerobic and anaerobic spores, especially those injured by dry heat or ethylene oxide. Efforts were concentrated to obtain a test organism whose spores require a heat shock for maximal germination. With spores of B. subtilis var. niger, which are used in many of our model systems, heat shocking at 80 C for 15 minutes consistently resulted in viable counts one-third to one-half those obtained from suspensions not so treated. On the other hand, when spores of B. subtilis strain 5230 were heat-shocked under the same conditions, resulting viable counts were two to three times higher than those obtained from suspensions that were not heat-shocked (Table 15). Consequently, the latter organisms will be employed for experiments to determine if supplements such as calcium dipicolinate can replace heat shock for spores injured by dry heat or ethylene oxide.

7. A research laboratory was established at Cape Kennedy for monitoring microbial contamination within areas used for the asembly, testing, and fueling of spacecraft. This laboratory has been equipped and staffed. Various spacecraft contractors were contacted in order to explain the sampling program and to obtain their cooperation. Preliminary studies were initiated in Hangar S (Lunar Orbitor Assembly and Testing Area), Building AE, and a portable vertical laminar flow clean room within Building AE (Interplanetary Monitoring Platform Assembly and Testing Area), the Explosive Safe Facility (Surveyor fueling area) and the operating suite of the Bioastronautics Hospital (Figures 10, 11, 12, 13). Results obtained during the first few weeks of sampling showed a relatively low level of airborne microorganisms accumulating on stainless steel strips in most of the areas which had little or no activity (Tables 16 and 17). In Hangar S there was no activity in the areas tested during the first two weeks. During the third week of sampling, the prototype spacecraft and electrical equipment were moved into the clean room. During this latter period there was heavy personnel activity with as many as 15 people present in the room at one time. This increased activity was accompanied by a sharp increase in the levels of microorganisms accumulating on stainless steel strips (Table 18). More definitive information will be available during the next quarter.

TABLE 1. EFFECT OF PLATING TECHNIQUE ON DEVELOPMENT OF VISIBLE COLONIES OF *B. SUBTILIS* VAR. *NIGER* ON DIFFERENT TYPES OF MEMBRANE FILTERS.

Type of filter	Hours of incubation at 32 C	Method[2]		
		Filter placed on surface of TSA[1] No. of colonies	Filter overlaid with TSA No. of colonies	Filter placed on molten TSA No. of colonies
Millipore, HA 0.45 micron	24	353	0	331
	48	-	-	-
	72	-	268	-
Gelman, GA 0.45 micron	24	315	0	346
	48	-	48	-
	72	-	231	-
Gelman, Alpha 6 0.45 micron; solvent resistant	24	169	49	216
	48	-	196	313

[1] Trypticase Soy Agar

[2] Spores were suspended in carbon tetrachloride and aliquots passed through each type of filter.

TABLE 2. RECOVERY OF SPORES OF B. SUBTILIS VAR. NIGER FROM COTTON SWABS BY MECHANICAL AGITATION AND ULTRASONICATION.

Test No.	Procedure	Spores recovered per $in.^2$	Coefficient of variation %	Probability factor
1	Ultrasonic bath	1,412	8.1	$P < 0.001$
	Mechanical agitation	1,094	9.8	
2	Ultrasonic bath	1,047	4.1	$P < 0.20$
	Mechanical agitation	910	22.0	
3	Ultrasonic bath	214	7.2	$P < 0.001$
	Mechanical agitation	151	19.5	
4	Ultrasonic bath	146	10.1	$P < 0.02$
	Mechanical agitation	98	35.6	

TABLE 3. RECOVERY OF MICROBIAL CONTAMINATION FROM COTTON SWABS BY MECHANICAL AGITATION AND ULTRASONICATION.

Test No.	Procedure	Microorganisms[1] recovered per in.[2]	Coefficient of variation %	Probability factor
1	Ultrasonic bath	71	70.7	$P < 0.001$
	Mechanical agitation	33	73.0	
2	Ultrasonic bath	21	54.8	$P < 0.05$
	Mechanical agitation	18	87.2	

[1] Natural contamination on floor of laboratory

TABLE 4. COMPARISON OF TWO ULTRASONIC BATHS IN THE REMOVAL OF HEAT-FIXED SPORES OF B. SUBTILIS VAR. NIGER FROM STAINLESS STEEL AND GLASS STRIPS.

Ultrasonic bath	Rated power in watts	Percentage removal
	STAINLESS STEEL	
JPL	150	93.6
CDC	150	63.7
CDC	300	88.3
	GLASS	
JPL	150	84.6
CDC	150	48.7
CDC	300	78.6

TABLE 5. COMPARISON OF MECHANICAL AGITATION AND THREE ULTRASONIC SYSTEMS IN REMOVING SPORES OF *B. SUBTILIS* VAR. *NIGER* FROM STAINLESS STEEL AND GLASS STRIPS.

	System	Surface	Coefficient of variation (%)	Percentage of recovery
A.	Mechanical agitation	Stainless steel	4.4	94.2
		Glass	14.0	67.1
B.	Ultrasonic probe	Stainless steel	2.2	96.1
		Glass	2.1	93.5
C.	JPL unit; Ultrasonic bath, 150 watts	Stainless steel	7.9	95.6
		Glass	18.8	84.1
D.	CDC unit; Ultrasonic bath, 300 watts	Stainless steel	6.1	96.2
		Glass	2.6	98.0

TABLE 6. A COMPARISON OF MECHANICAL AGITATION AND ULTRASONICATION IN REMOVING SPORES OF B. SUBTILIS VAR. NIGER FROM SURFACES OF STAINLESS STEEL AND FROSTED GLASS.

Surface	Pretreatment of inoculated surface	Percentage removal	
		Mechanical agitation	Ultrasonication
Stainless steel	none	95	99
Stainless steel	heat[1]	91	91
Frosted glass	none	26	92
Frosted glass	heat[1]	13	96

[1] Inoculated test surfaces were placed in a dry heat oven at 120 C for 20 minutes.

TABLE 7. COMPARATIVE RATES OF RECOVERY OF B. SUBTILIS VAR. NIGER SPORES FROM SURFACES PLACED ONTO OR SUSPENDED FROM THE BOTTOM OF AN ULTRASONIC BATH.

Surface	Pretreatment of inoculated surface	Percentage recovery[1]	
		Suspended 1 in. from tank bottom	Resting on tank bottom
Stainless steel	none	77	97
Stainless steel	heat[2]	54	96
Frosted glass	none	74	92
Frosted glass	heat[2]	67	98

[1] Mean of 5 samples

[2] Inoculated test surfaces were placed in a dry heat oven at 120 C for 20 minutes.

TABLE 8. COATED[1] AND UNCOATED STAINLESS STEEL STRIPS RETAINING MICROBIAL CONTAMINATION[2] AFTER MECHANICAL AGITATION IN PEPTONE WATER.

	Coated	Uncoated
Strips plated	102	102
Strips with colonies	5	41
Percent with colonies	4.9	40.2

1 Sodium silicate

2 Strips were contaminated as the result of fallout of airborne microorganisms.

TABLE 9. RECOVERY OF MICROORGANISMS FROM COATED[1] AND UNCOATED STAINLESS STEEL STRIPS CONTAMINATED BY HANDLING.

	Experiment 1	Experiment 2
Coated strips handled	30	40
Mean % recovery	99.7	98.9
Uncoated strips handled	13	10
Mean % recovery	85.4	91.4
Probability factor	$P < .001$	$P < .001$

[1] Sodium silicate

TABLE 10. RECOVERY OF HEAT-FIXED SPORES FROM COATED[1] AND UNCOATED STAINLESS STEEL STRIPS CONTAMINATED BY SIMULATED AERIAL FALLOUT[2].

	Experiment 1	Experiment 2
Coated strips exposed	12	12
Mean % recovery	99.9	99.6
Uncoated strips exposed	12	12
Mean % recovery	94.3	93.8
Probability factor	$P < .001$	$P < .001$

1 Sodium silicate

2 Strips were exposed to an aerosol, B. subtilis var. niger in an aerosol chamber.

TABLE 11. RECOVERY OF MICROORGANISMS FROM GLASS SURFACES COATED WITH SODIUM SILICATE.

Test number and micro-organism	Condition of surface	Pretreatment of inoculated surface	Percentage recovery[1]	Probability factor
1. *B. subtilis* var. *niger*	uncoated	none	96.6	$P < 0.02$
	coated	none	99.9	
	uncoated	heat	97.6	$P < 0.05$
	coated	heat	99.8	
2. *B. subtilis* var. *niger*	uncoated	none	88.3	$P < 0.01$
	coated	none	94.9	
	uncoated	heat	88.3	$P < 0.02$
	coated	heat	94.9	
3. Natural contamination as the result of handling	uncoated	none	98.7	$P < 0.02$
	coated	none	97.3	

[1] Mean of 10 samples

TABLE 12. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL STRIPS EXPOSED WITHIN HOSPITAL A.

Weeks of exposure	Samples not heat-shocked		Samples heat-shocked at 80 C for 15 min.	
	Aerobes No./ft^2	Anaerobes No./ft^2	Aerobes No./ft^2	Anaerobes No./ft^2
	OPERATING ROOM 1			
1	28,260	4,402	2,938	1,800
2	56,102	4,320	3,240	1,260
3	41,278	5,278	5,098	3,838
4	69,357	5,580	4,320	3,398
5	44,784	4,500	2,664	1,858
6	22,176	5,580	3,672	1,922
7	27,504	7,978	6,660	3,658
8	43,502	6,718	2,462	1,318
	OPERATING ROOM 2			
3	68,112	9,180	2,664	1,858
6	31,500	5,940	3,298	1,440
9	49,442	6,538	4,918	1,980
12	22,558	3,542	6,300	3,298
15	20,376	3,535	7,135	2,520
18	21,960	6,898	5,976	2,815
21	111,895[1]	7,078	6,178	2,275

[1] The tray containing stainless steel strips was knocked over prior to assay.

TABLE 13. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL STRIPS EXPOSED WITHIN HOSPITAL B.

Weeks of exposure	Samples not heat-shocked		Samples heat-shocked at 80 C for 15 min.	
	Aerobes No./ft^2	Anaerobes No./ft^2	Aerobes No./ft^2	Anaerobes No./ft^2
	OPERATING ROOM 1			
1	61,078	6,782	2,462	2,462
2	39,362	11,700	3,542	1,022
3	17,942	5,278	1,318	1,022
4	19,894	6,120	4,622	1,022
5	28,742	3,092	3,658	1,922
6	25,380	4,918	6,214	2,520
	OPERATING ROOM 2			
3	19,620	13,018	1,678	1,260
6	20,880	4,982	2,160	1,440
9	18,720	3,780	4,622	2,218
12	23,278	3,730	5,162	2,038
15	159,480[1]	70,560	151,798	61,380
18	56,758	43,618	42,718	30,960

[1] A new floor was put in during the 12th and 15th weeks of exposure. This activity lasted 2 to 3 days.

TABLE 14. TYPES OF AEROBIC MESOPHILIC MICROORGANISMS WHICH ACCUMULATED ON STAINLESS STEEL SURFACES EXPOSED TO THE INTRAMURAL AIR OF 2 OPERATING ROOMS, 1 INDUSTRIAL CLEAN ROOM AND 1 FACTORY AREA.

Type of microorganism	Hospital A; O.R. 2 %	Hospital B; O.R. 2 %	Clean Room B %	Factory Area C %
Staphylococcus aureus	0	0	0.5	0
Staphylococcus epidermidis	16.2	13.4	47.2	12.7
Micrococcus spp.	15.8	7.4	6.4	1.6
Streptococcus spp.	0.4	0	0.9	0
Gaffkya spp.	1.6	1.0	0	0
Sarcina spp.	0.4	0.5	0	0
Gram negative microorganisms	5.5	2.0	0	15.1*
Bacillus spp. (sporeformers)	26.3	41.6	10.1	30.9
Corynebacterium-Brevibacterium group	10.5	8.4	28.4	10.3
Yeasts	0.4	0.5	0.9	1.6
Molds	16.6	19.8	6.4	25.4
Actinomycetes and Streptomycetes	7.3	5.4	1.4	2.4
Unidentified or lost upon subculture	0	0	1.4	0

* Pseudomonas spp.

TABLE 15. EFFECT OF HEAT SHOCKING SPORES OF *B. SUBTILIS* VAR. *NIGER* AND *B. SUBTILIS* 5230.

Microorganism	No heat shock	Heat shocked at 80 C for 15 min.
	No./ml x 10^5	No./ml x 10^5
B. subtilis var. *niger*		
1. Water suspension F-W	3,700	1,620
2. Ethanol suspension F-A[1]	4,350	1,670
3. Ethanol suspension G-A	1,100	355
B. subtilis 5230		
1. Water suspension NAS-W	385	925
2. Ethanol suspension NAS-A	280	840

[1] Spore suspensions which were stored in 95 percent ethanol were diluted in sterile distilled water before each test.

TABLE 16. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL STRIPS EXPOSED TO THE INTRAMURAL ENVIRONMENT OF AREAS USED FOR THE ASSEMBLY AND TEST OF THE SURVEYOR SPACECRAFT.

Area[1]	Weeks of exposure	Samples not heat-shocked		Samples heat-shocked at 80 C for 15 min.	
		Aerobes No./ft^2	Anaerobes No./ft^2	Aerobes No./ft^2	Anaerobes No./ft2
Building AO (Conventional clean room)					
Site 1.					
South end of platform	1	0	58	0	0
in clean room	2	122	0	0	58
Site 2.					
North end of platform	1	0	58	0	0
in clean room	2	360	0	0	0
Explosive Safe Facility Surveyor Fueling Area (No special environmental control)					
Site 1.	1	720	122	0	0
Site 2.	1	302	302	58	0

[1] All of the areas were unoccupied during these preliminary tests except for janitorial personnel.

TABLE 17. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL STRIPS EXPOSED TO THE INTRAMURAL ENVIRONMENT OF AREAS USED FOR THE ASSEMBLY AND TEST OF THE INTERPLANETARY MONITORING PLATFORM.

Area	Weeks of exposure	Samples not heat-shocked		Samples heat-shocked at 80 C for 15 min.	
		Aerobes No./ft^2	Anaerobes No./ft^2	Aerobes No./ft^2	Anaerobes No./ft^2
Building AE; horizontal laminar flow clean room. Light personnel activity					
Site 1.					
3 feet from filter wall	1	0	0	0	0
	3	0	0	58	0
Site 2.					
Center of clean room	1	0	0	0	0
	3	122	0	0	0
Site 3.					
2 feet from exhaust wall	1	2.9×10^4	58	0	0
	3	0	0	0	0
Portable vertical laminar flow clean room within the cleanroom above. Spacecraft in room. Light personnel activity.					
Site 1.	1	58	0	0	0
	2	0	0	0	0

TABLE 18. LEVELS OF MICROBIAL CONTAMINATION WHICH ACCUMULATED ON STAINLESS STEEL STRIPS EXPOSED TO THE INTRAMURAL ENVIRONMENT OF AREAS USED FOR THE ASSEMBLY AND TEST OF THE LUNAR ORBITOR.

Area	Weeks of exposure	Samples not heat-shocked		Samples heat-shocked at 80 C for 15 min.	
		Aerobes No./ft^2	Anaerobes No./ft^2	Aerobes No./ft^2	Anaerobes No./ft^2
Hangar S; conventional clean room					
1st Series*					
Site 1	2	93	58	122	58
	3	0	0	122	0
Site 2	2	0	0	237	58
	3	0	58	0	58
2nd Series*					
Site 1	1	763	1,138	58	58
Site 2	1	58	0	208	0
	2	2,642	360	0	0

* There was no activity in these areas during the first 2 weeks (1st series). During the third week, the prototype spacecraft and electrical equipment were moved into the room. At the end of the third week the flight spacecraft was moved into the room. The old trays containing stainless steel strips were replaced with new ones (2nd series). During the first week there was heavy activity with as many as 15 people in the room at one time.

LEGEND FOR FIGURES

1. Comparative survival rates of spores of Bacillus subtilis var. niger on polystyrene at temperatures of 20, 37, and 50 C, and at a relative humidity (R.H.) of 46 percent.
2. Survival rates of spores of B. subtilis var. niger on granules of polystyrene at 37 C and 46 and 0 percent R.H.
3. Survival rates of spores of B. subtilis var. niger on granules of polystyrene at 37 C and 46 and 0 percent R.H.
4. Survival of a water suspension of spores of B. subtilis var. niger on stainless steel surfaces at 50 C and 46 and 0 percent R.H.
5. Survival of an ethanol suspension of spores of B. subtilis var. niger on stainless steel surfaces at 50 C and 46 and 0 percent R.H.
6. Comparative levels of airborne microbial contamination which accumulated on stainless steel strips exposed to the intramural environments of two hospital operating rooms, a manufacturing area, and a conventional clean room for 21 weeks.
7. Levels of airborne contamination which accumulated on stainless steel strips exposed to the intramural environments of two hospital operating rooms for six and eight weeks.
8. Levels of airborne contamination in operating room 2 of Hospital B.
9. Comparative levels of airborne contamination in an operating room, a factory area, a conventional clean room (A), and a horizontal laminar flow clean room (D).
10. Areas used for the assembly and testing of lunar spacecraft.
11. Hangar "S".
12. Building AO.
13. Building AE.

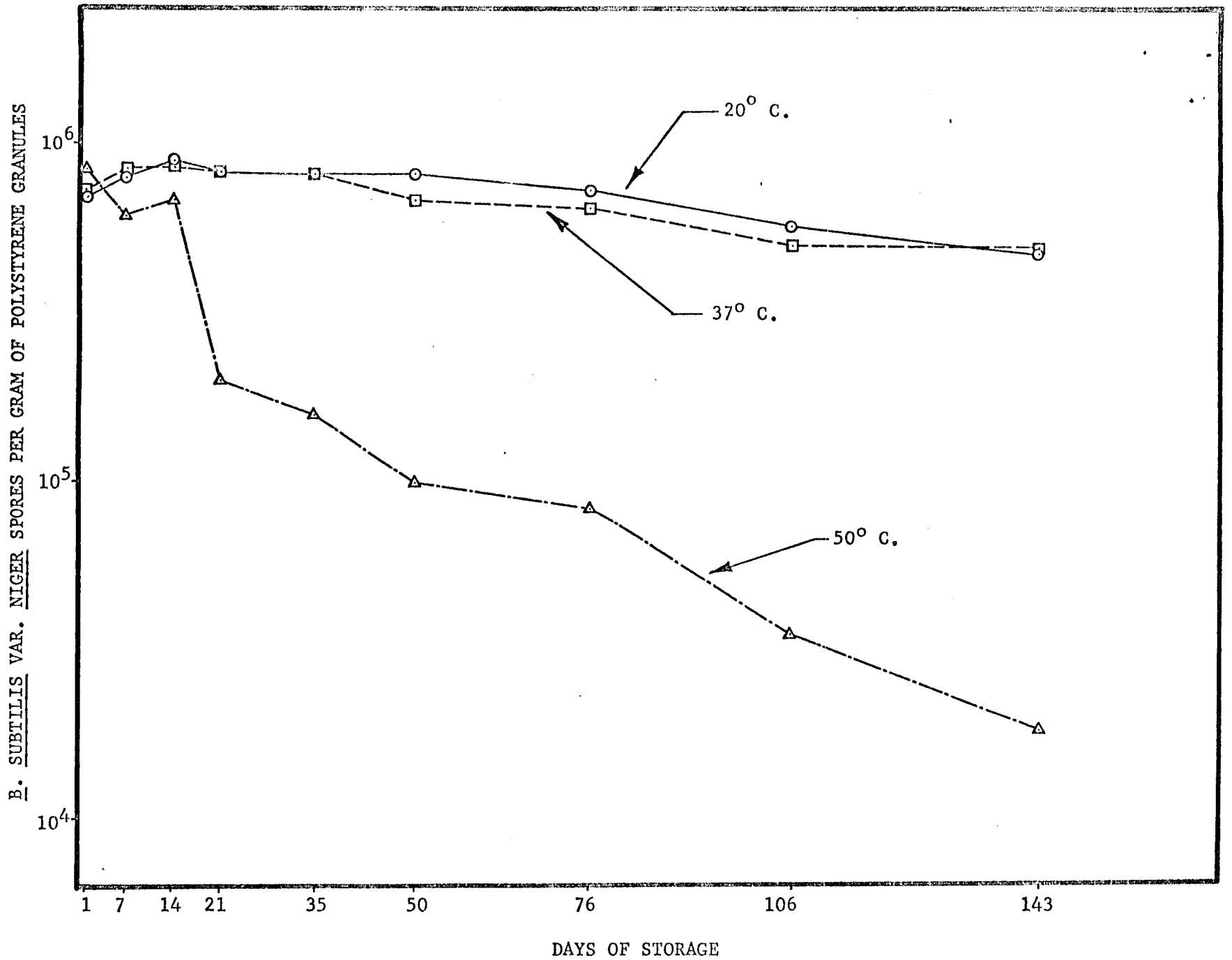

B. SUBTILIS VAR. NIGER SPORES PER GRAM OF POLYSTYRENE GRANULES
10^6
10^5
10^4
20° C.
37° C.
50° C.
1
7
14
21
35
50
76
106
143
DAYS OF STORAGE

FIGURE 1.

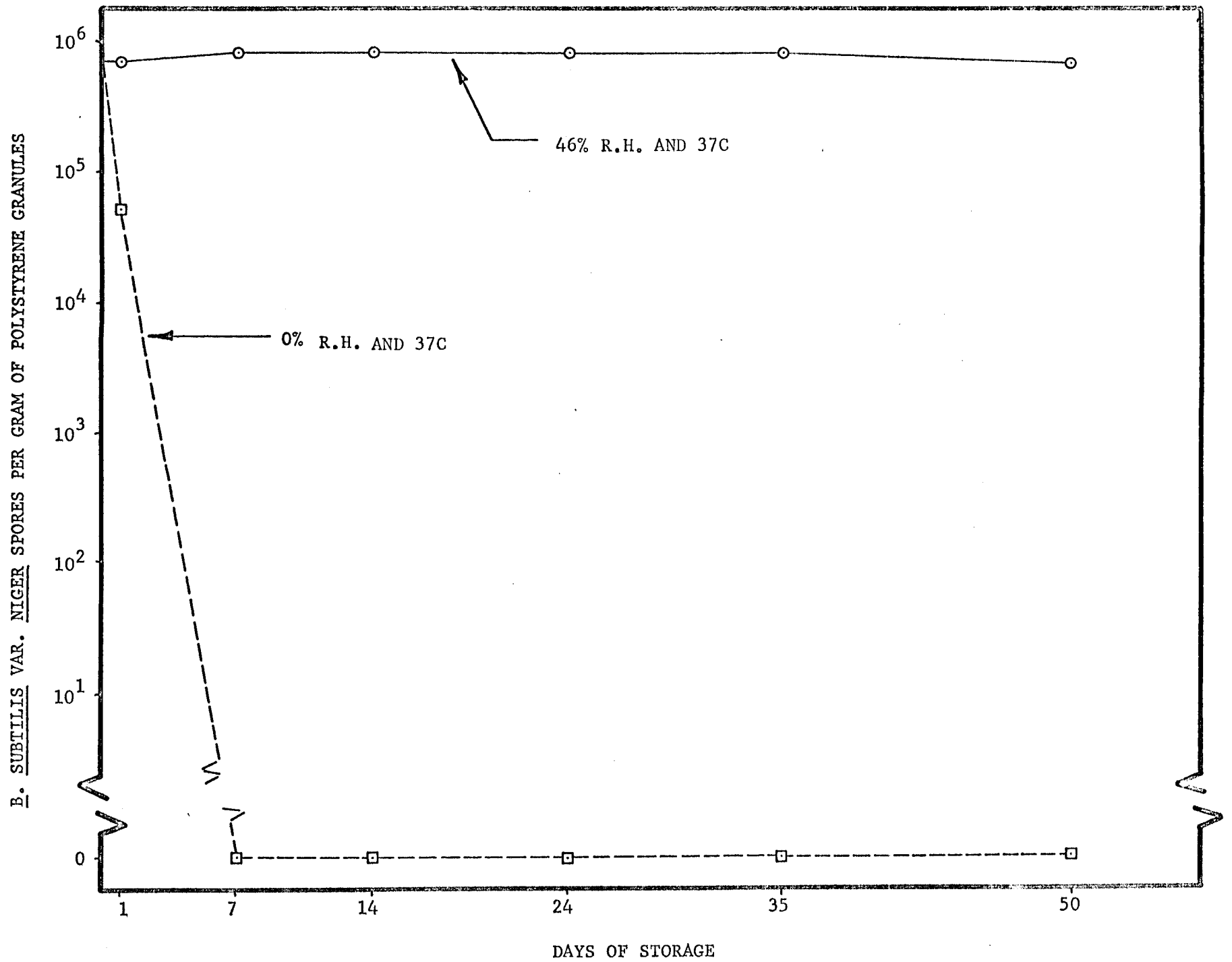

B. SUBTILIS VAR. NIGER SPORES PER GRAM OF POLYSTYRENE GRANULES
10^6
10^5
10^4
10^3
10^2
10^1
0
46% R.H. AND 37C
0% R.H. AND 37C
1
7
14
24
35
50
DAYS OF STORAGE

FIGURE 2.

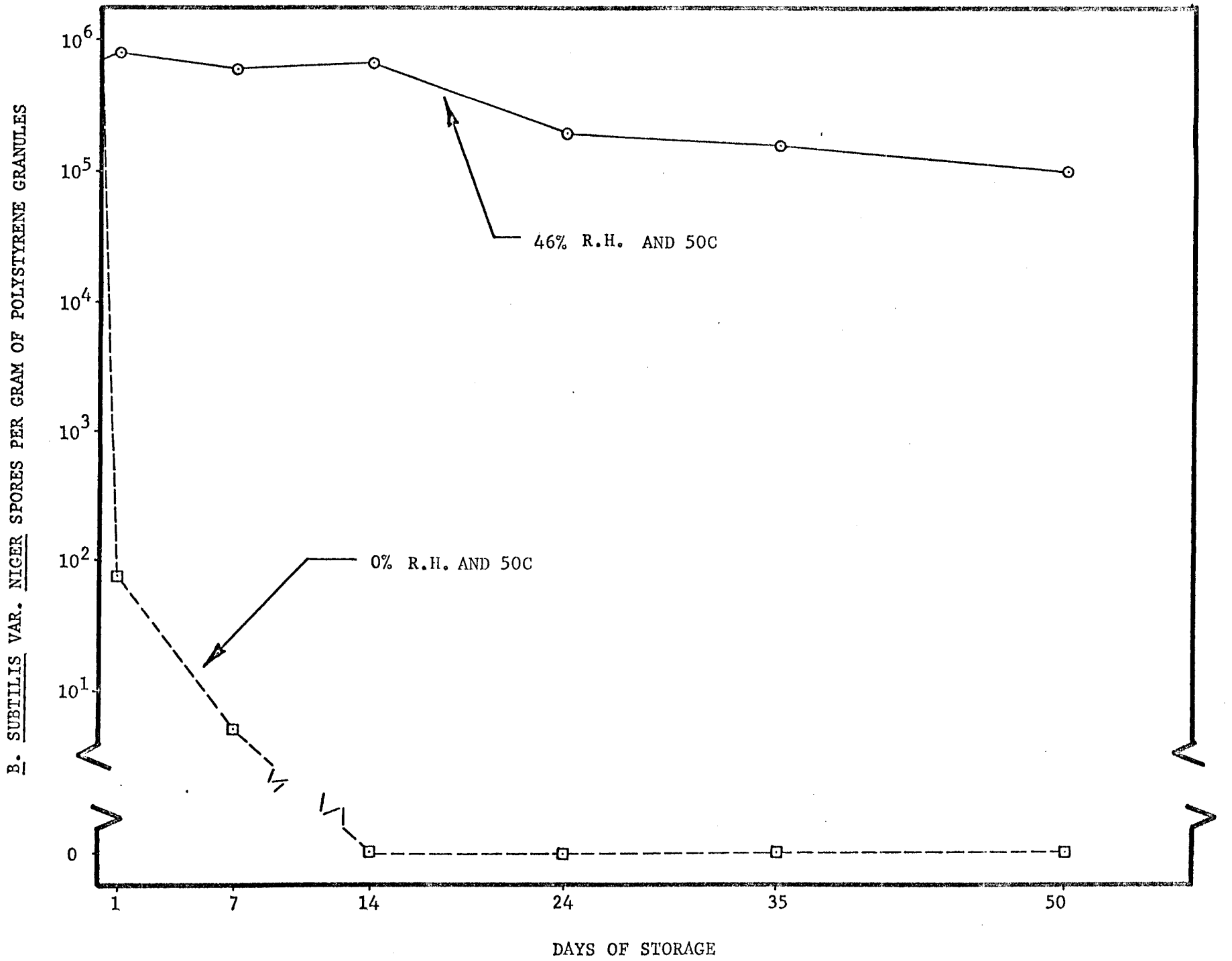

B. SUBTILIS VAR. NIGER SPORES PER GRAM OF POLYSTYRENE GRANULES
10^6
10^5
10^4
10^3
10^2
10^1
0
46% R.H. AND 50C
0% R.H. AND 50C
1
7
14
24
35
50
DAYS OF STORAGE

FIGURE 3.

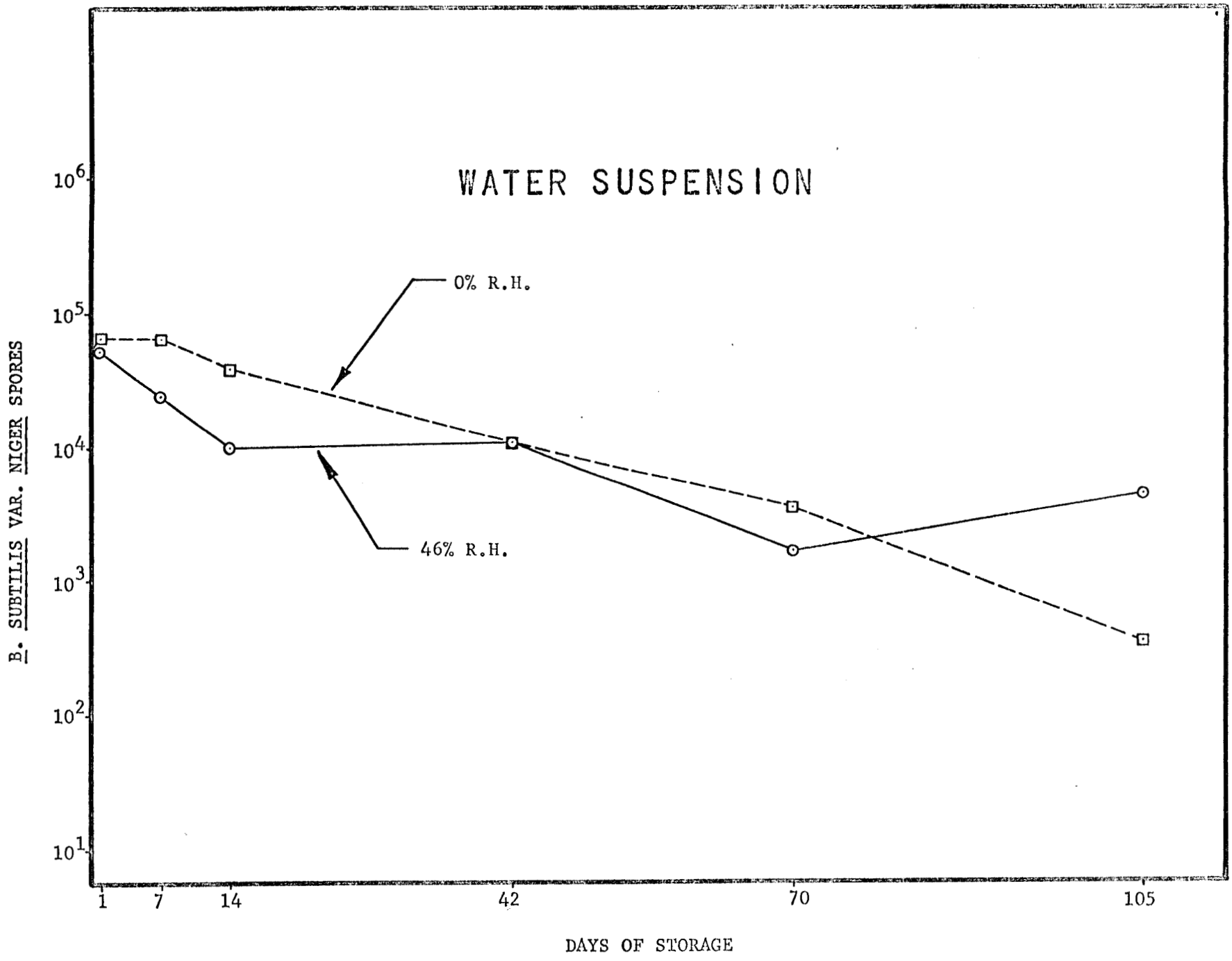

WATER SUSPENSION
0% R.H.
46% R.H.
B. SUBTILIS VAR. NIGER SPORES
10^6
10^5
10^4
10^3
10^2
10^1
1
7
14
42
70
105
DAYS OF STORAGE

FIGURE 4.

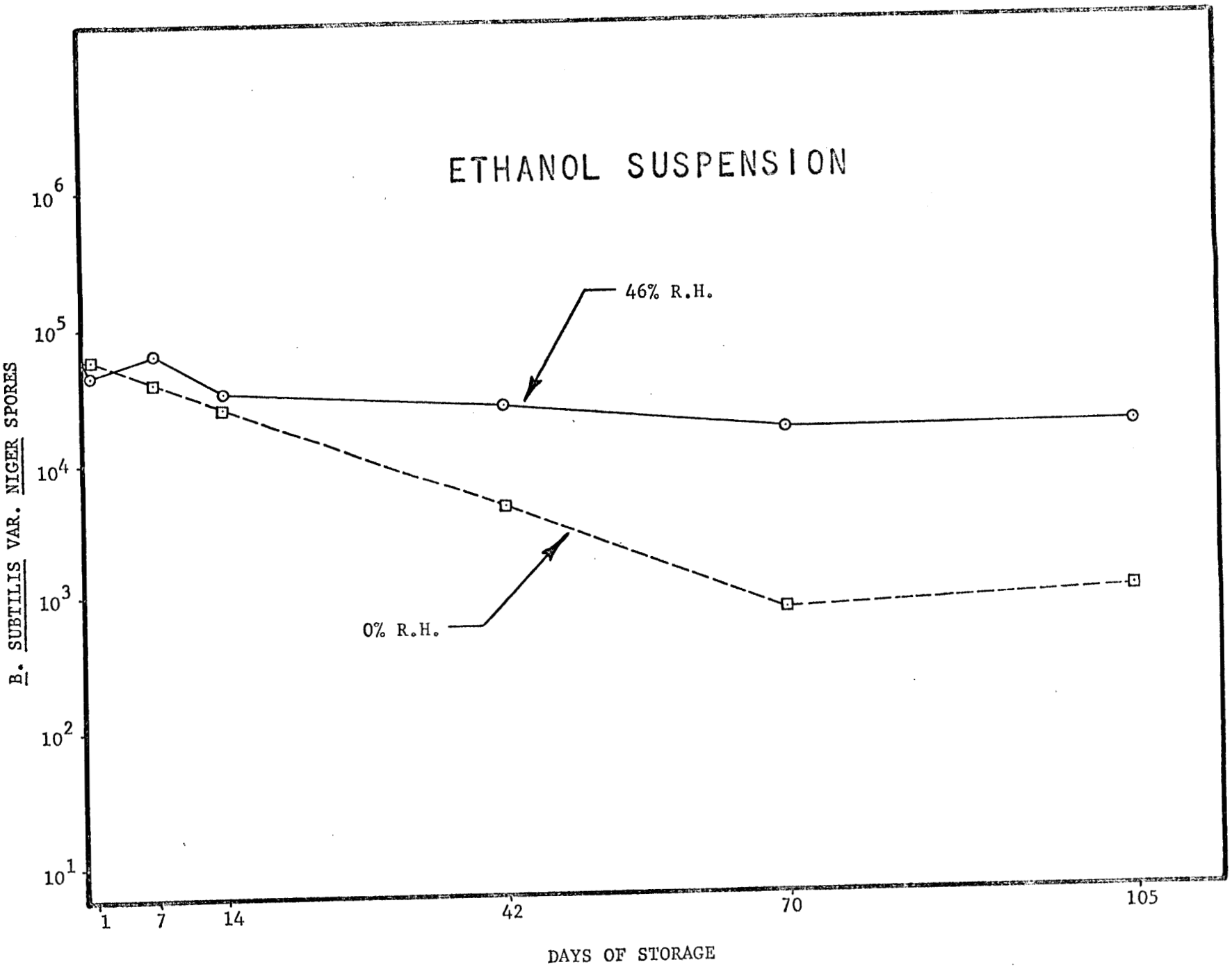

ETHANOL SUSPENSION
B. SUBTILIS VAR. NIGER SPORES
10^6
10^5
10^4
10^3
10^2
10^1
46% R.H.
0% R.H.
1
7
14
42
70
105
DAYS OF STORAGE

FIGURE 5.

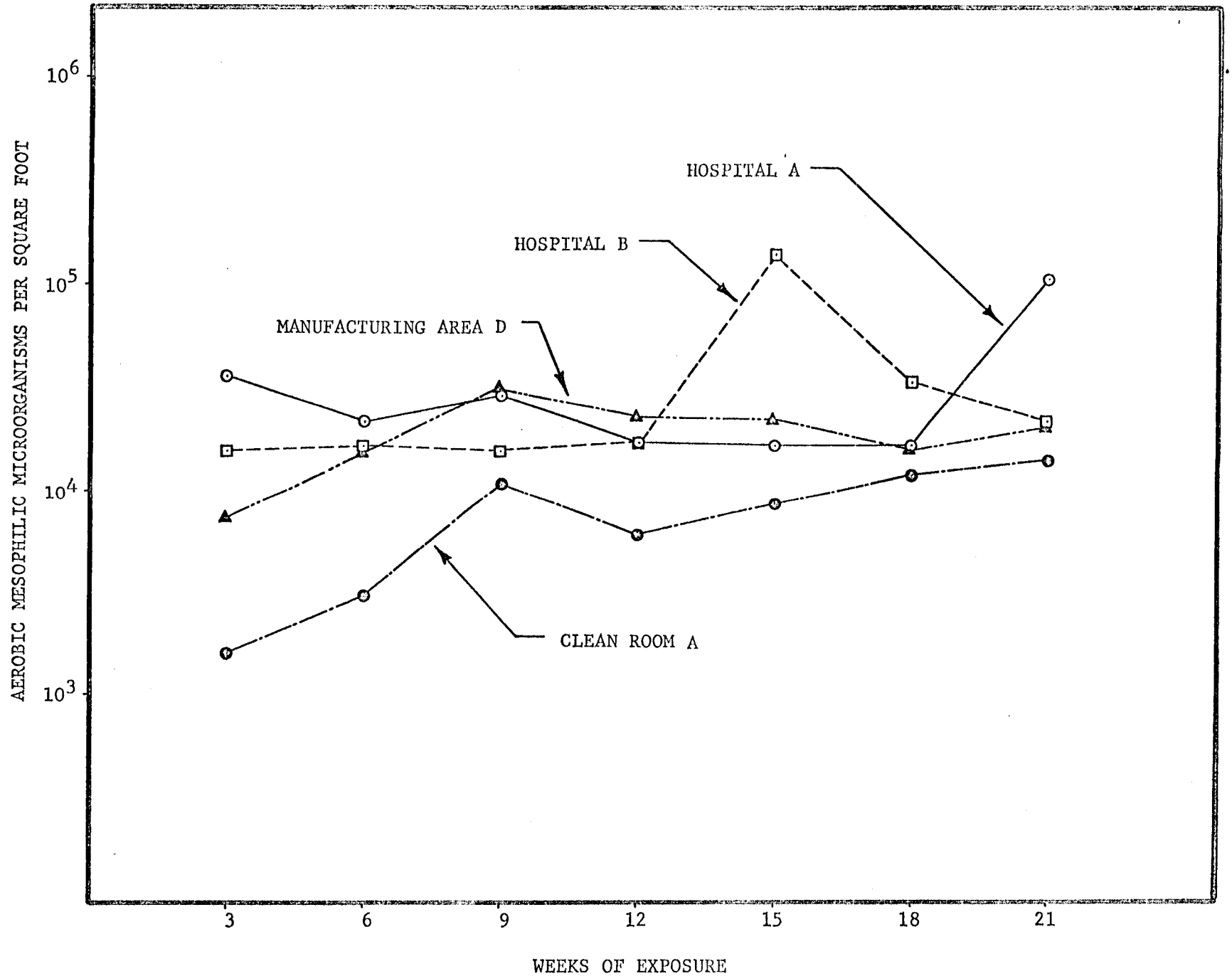

AEROBIC MESOPHILIC MICROORGANISMS PER SQUARE FOOT
10^6
10^5
10^4
10^3
HOSPITAL A
HOSPITAL B
MANUFACTURING AREA D
CLEAN ROOM A
3
6
9
12
15
18
21
WEEKS OF EXPOSURE

FIGURE 6.

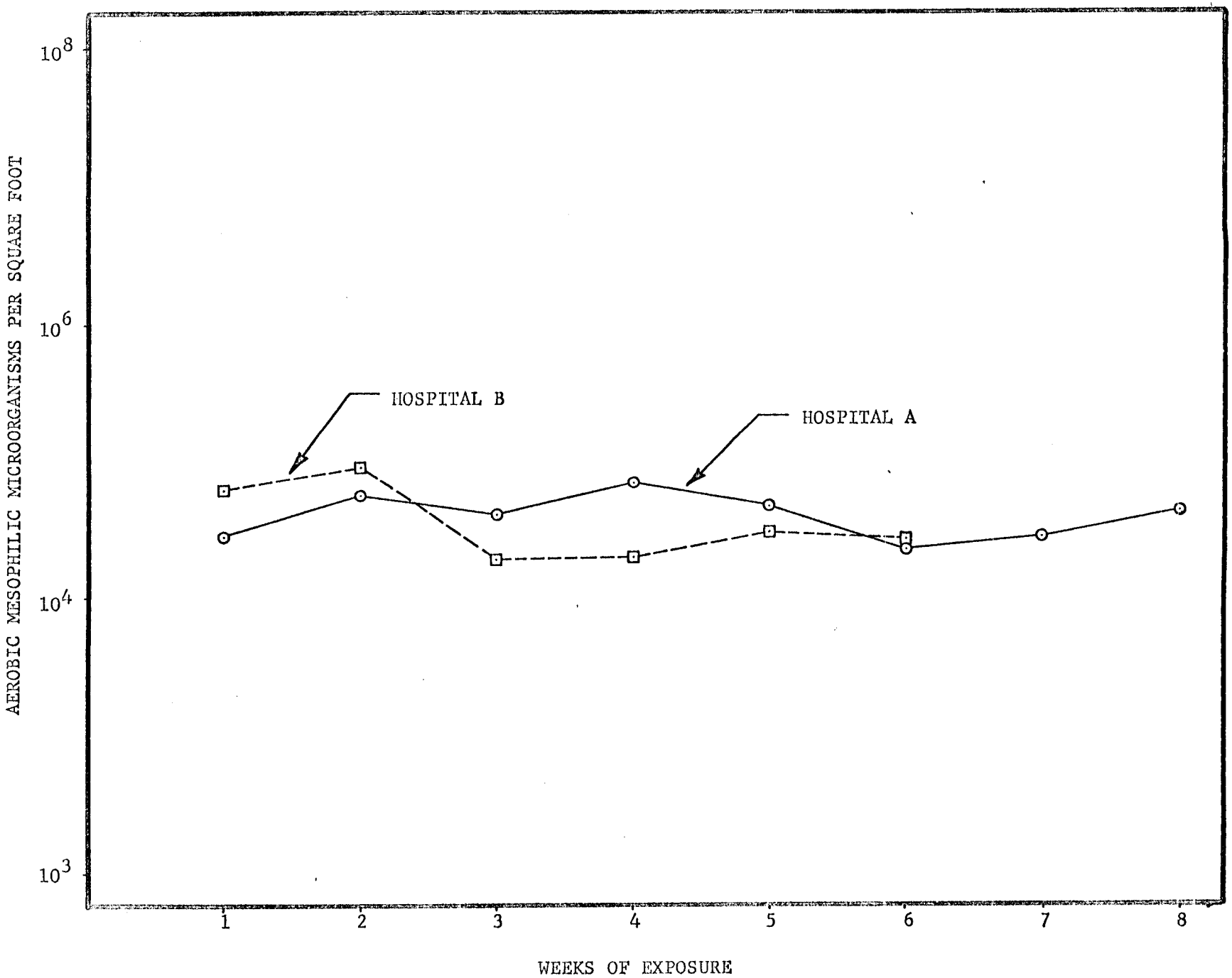

AEROBIC MESOPHILIC MICROORGANISMS PER SQUARE FOOT
10^8
10^6
10^4
10^3
HOSPITAL B
HOSPITAL A
1
2
3
4
5
6
7
8
WEEKS OF EXPOSURE

FIGURE 7.

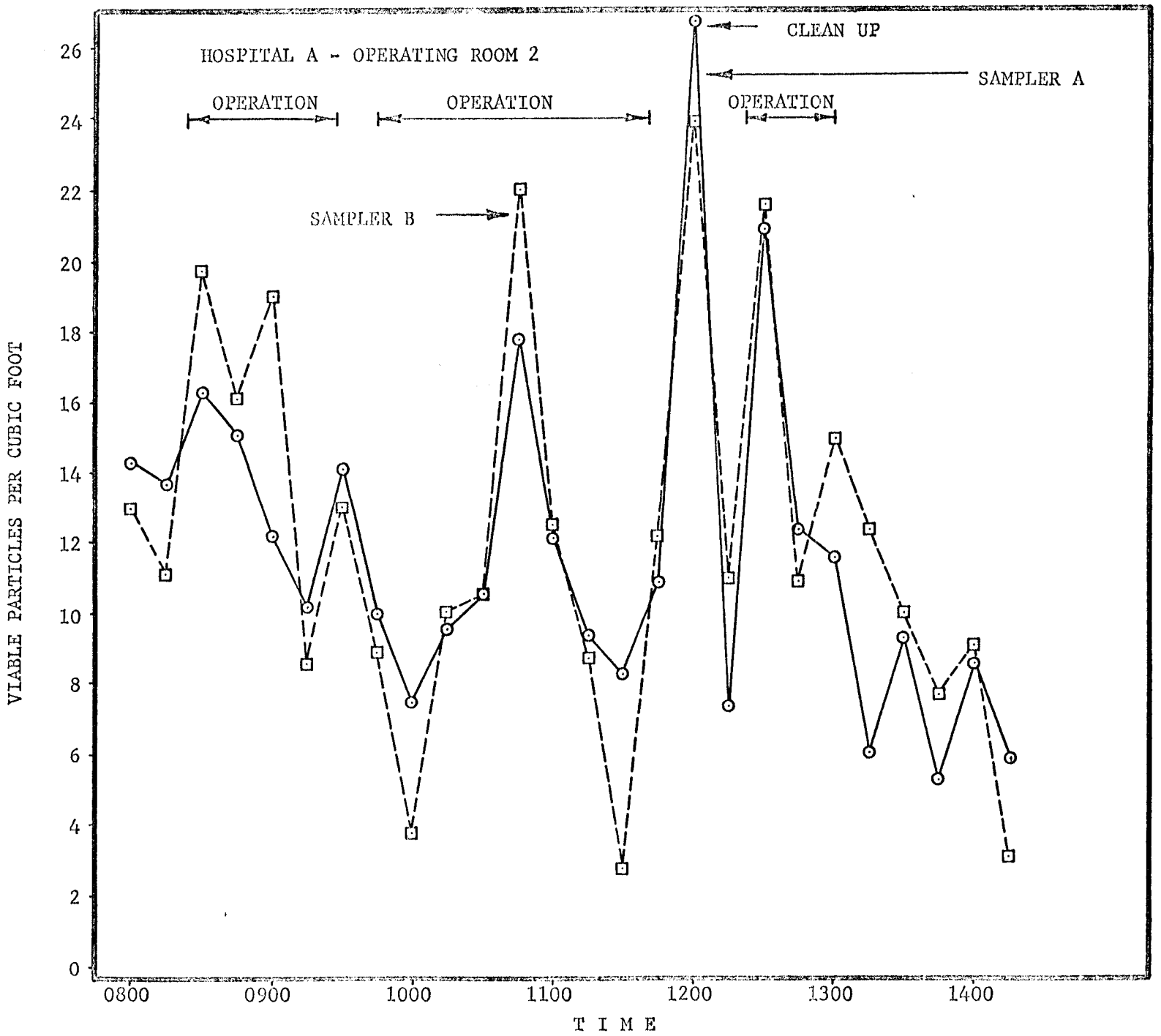

HOSPITAL A - OPERATING ROOM 2
CLEAN UP
SAMPLER A
OPERATION
OPERATION
OPERATION
SAMPLER B
VIABLE PARTICLES PER CUBIC FOOT
0
2
4
6
8
10
12
14
16
18
20
22
24
26
0800
0900
1000
1100
1200
1300
1400
T I M E

FIGURE 8.

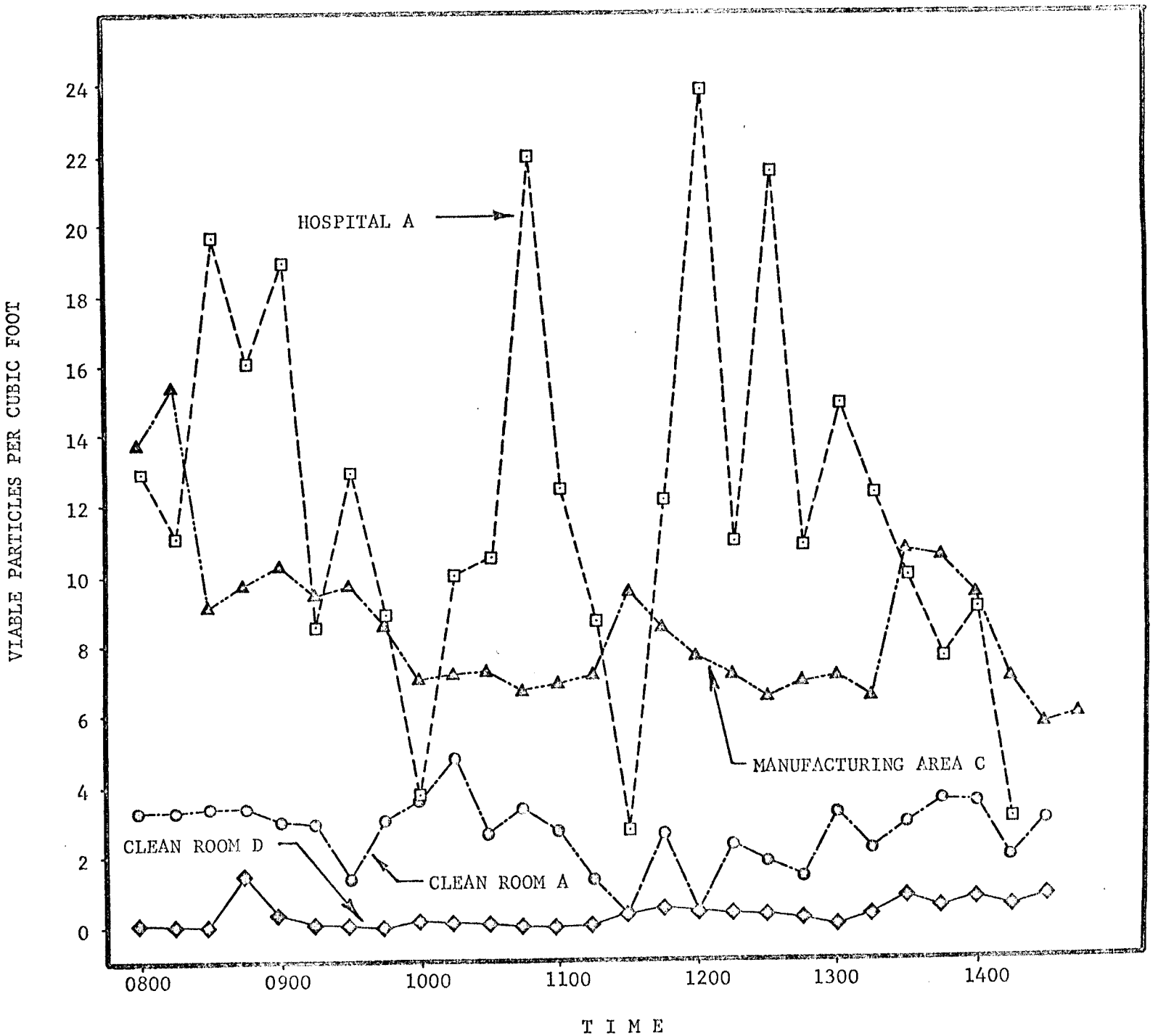

VIABLE PARTICLES PER CUBIC FOOT
24
22
20
18
16
14
12
10
8
6
4
2
0
HOSPITAL A
MANUFACTURING AREA C
CLEAN ROOM D
CLEAN ROOM A
0800
0900
1000
1100
1200
1300
1400
T I M E

FIGURE 9.

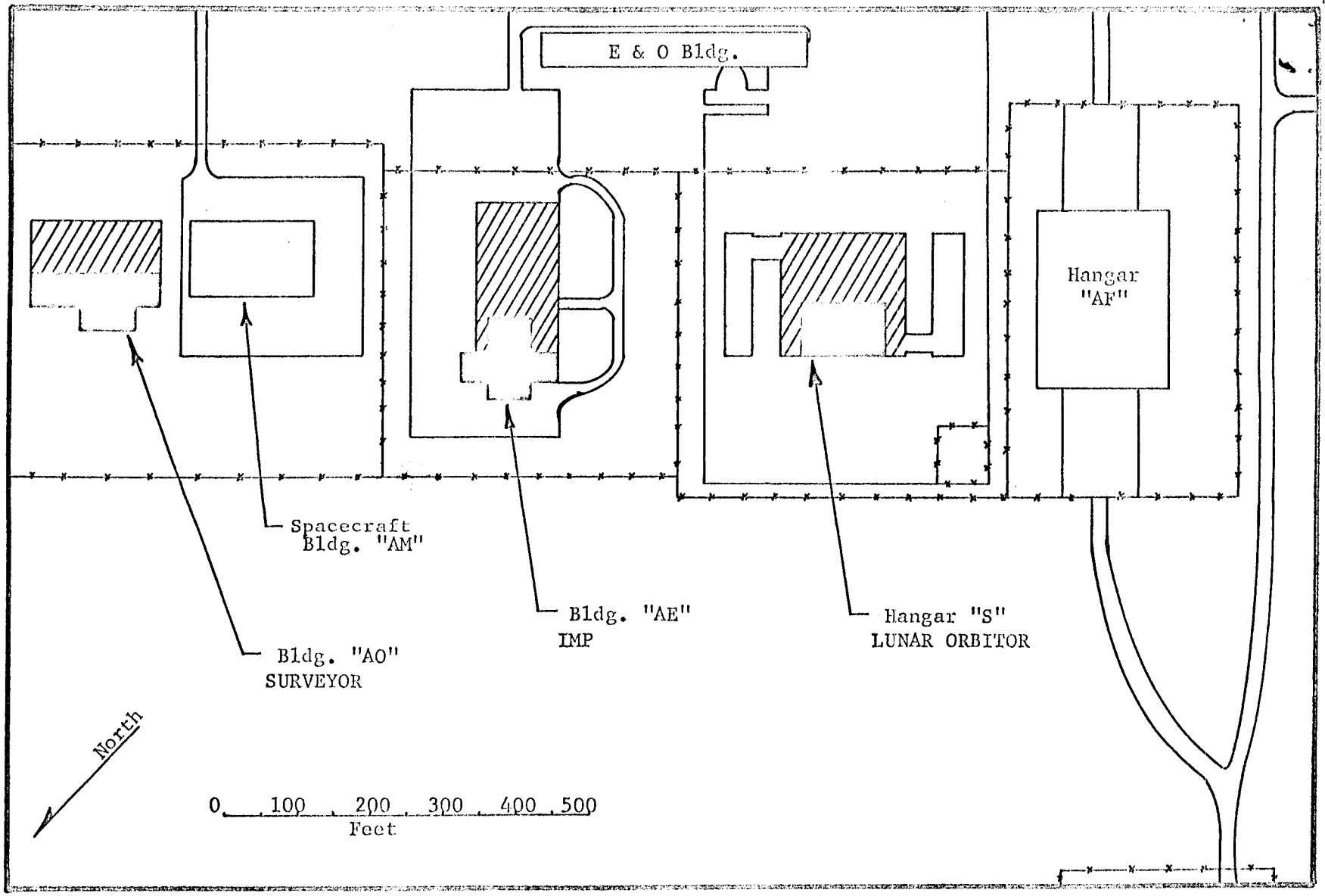
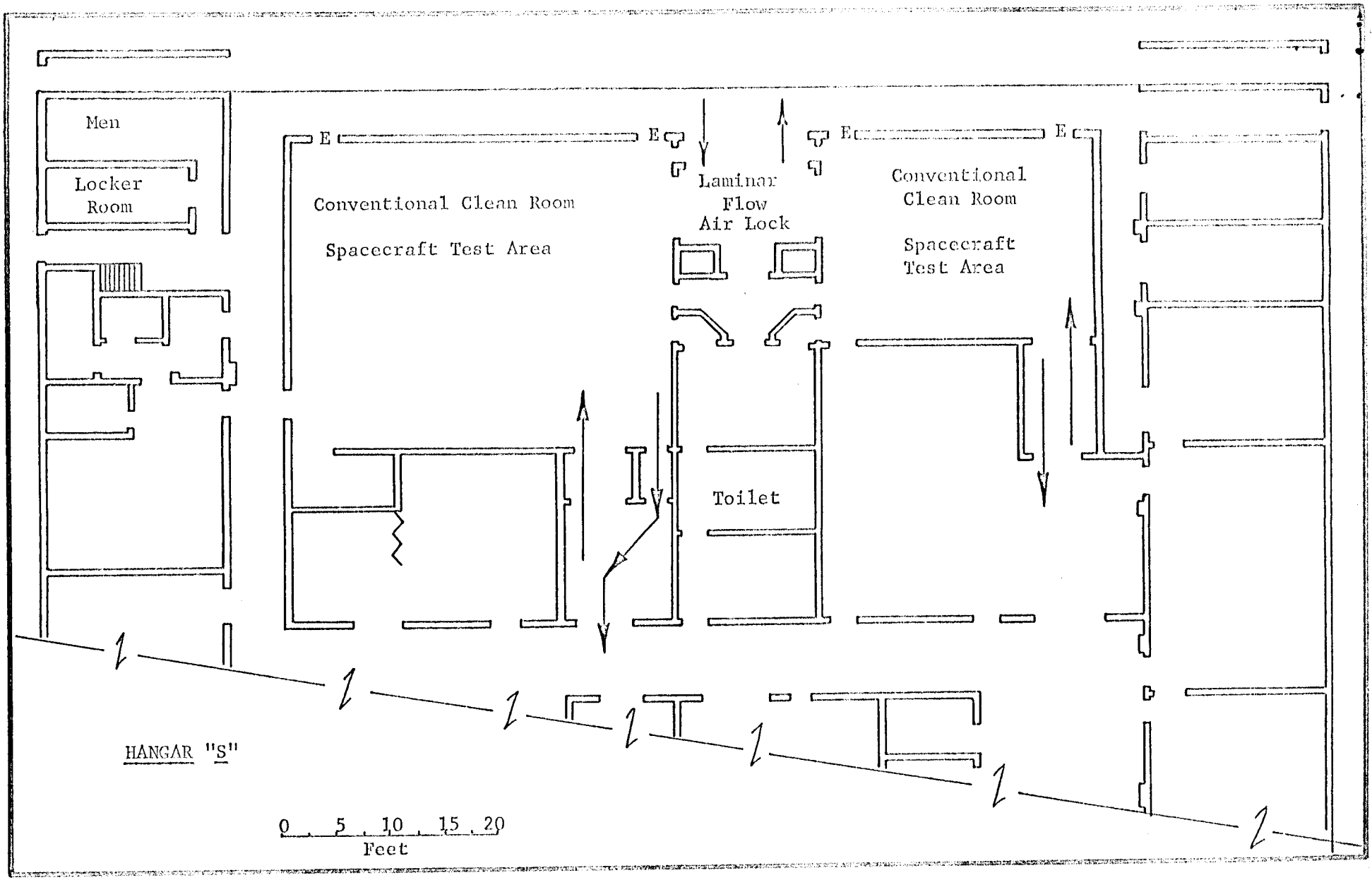
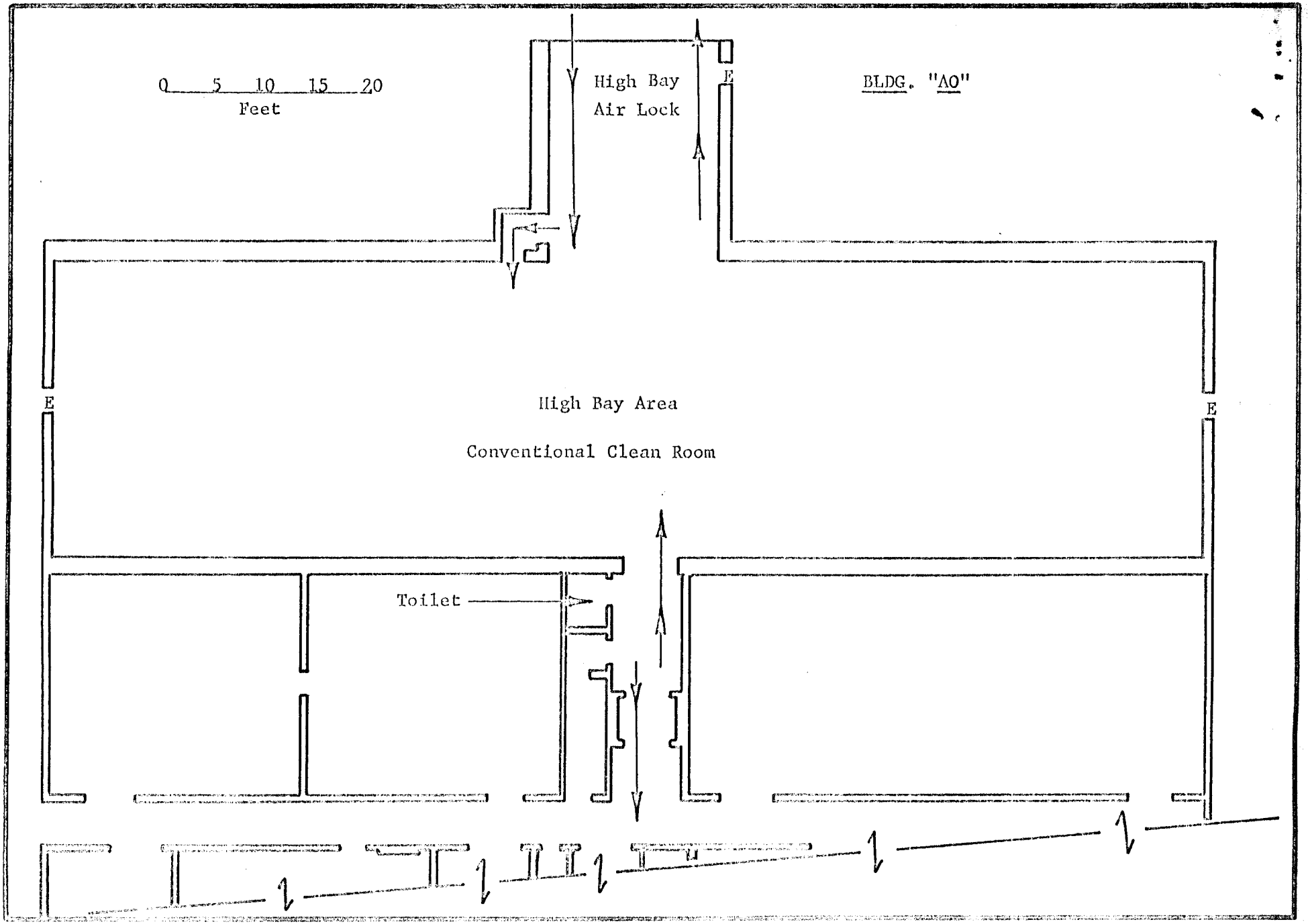

E & O Bldg.
Hangar
"AF"
Spacecraft
Bldg. "AM"
Bldg. "AE"
IMP
Hangar "S"
LUNAR ORBITOR
Bldg. "AO"
SURVEYOR
North
0 100 200 300 400 500
Feet

FIGURE 10.



ASSEMBLY AND TEST AREA FOR THE LUNAR ORBITOR

FIGURE 11.



ASSEMBLY AND TEST AREA FOR SURVEYOR

FIGURE 12.